

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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In re Patent Application of:  
Frank Loeffler

Application No.: 10/559,993

Confirmation No.: 7981

Filed: December 7, 2005

Art Unit: 1651

For: DEHALOCOCCOIDES ISOLATE FOR  
BIOREMEDIATION

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Examiner: D. K. Ware

**DECLARATION OF PROF. FRANK LOEFFLER, PH.D.**

MS AF  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

I, Frank Loeffler, hereby declare and state as follows:

1. I am a citizen of the Federal Republic of Germany and a permanent resident of the United States. I am more than twenty-one (21) years of age.

2. I am the named inventor in the above-identified patent application.

3. I have read and am familiar with the above-identified patent application as it was filed in the United States Patent and Trademark Office (the "PTO"). I have also read and am familiar with the pending claims of this application, as amended on July 3, 2008, as well as the Official Action mailed by the PTO on October 16, 2008.

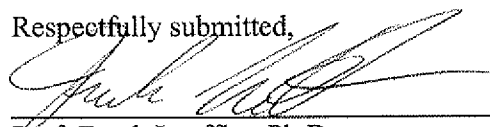
4. I am also a co-author, along with Jianzhong He, of an Abstract titled "Isolation Of a Vinyl Chloride-Respiring Population in Pure Culture," which was presented at the General Meeting of the American Society for Microbiology on or about May 18, 2003. For convenience, I refer to that abstract as the "He Abstract" throughout this declaration.

5. It is my understanding that the patent examiner has cited and relied upon the He Abstract to reject certain claims of this application. A copy of the abstract cited by the patent examiner is attached to this declaration, at Tab 1. I have reviewed the attached abstract at Tab 1, and confirm that it is a copy of the He Abstract co-authored by Dr. He and myself.

6. At the time the work described in the He Abstract was performed, Dr. He was a student working under my supervision and control and the Georgia Institute of Technology, School of Civil and Environmental Engineering. Dr. He contributed to the work described in the He Abstract by performing experiments under my direction. On information and belief, Dr. He did not contribute to conception of the presently claimed invention of this application, or make any other inventive contribution to that invention.

7. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true. I further declare that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States code, and that such willful false statements may jeopardize the validity of the this application or of any patent issuing therefrom.

Respectfully submitted,

  
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Prof. Frank Loeffler, Ph.D.

Dated: January 11, 2009

Attachment:

Tab 1: J. He and F. Loeffler, "Isolation of a Vinyl Chloride-Respiring Population in Pure Culture," *Abstracts of the General Meeting of the American Society for Microbiology* (May 18-22, 2003) Vol. 103, p. Q-016 (the "He Abstract").

# **TAB 1**

ANSWER 1 OF 4 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN  
AN 2003:532385 BIOSIS <<LOGINID::20081012>>  
DN PREV200300534302  
TI Isolation of a vinyl chloride-respiring population in  
pure culture.  
AU He, J. [Reprint Author]; Löffler, F. E. [Reprint Author]  
CS Georgia Institute of Technology, Atlanta, GA, USA  
SO Abstracts of the General Meeting of the American Society for Microbiology,  
(2003) Vol. 103, pp. Q-016.  
<http://www.asmta.org/mtgsrc/generalmeeting.htm>. cd-rom.  
Meeting Info.: 103rd American Society for Microbiology General Meeting.  
Washington, DC, USA. May 18-22, 2003. American Society for Microbiology.  
ISSN: 1060-2011 (ISSN print).  
DT Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LA English  
ED Entered STN: 12 Nov 2003  
Last Updated on STN: 12 Nov 2003

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CONTINUE? (Y)/N:y

L6 ANSWER 1 OF 4 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on  
STN

AB About half of the hazardous waste sites on the U.S. Environmental  
Protection Agency's Final National Priority List for 2002 were  
contaminated with tetrachloroethene (PCE) and/or trichloroethene (TCE).  
Dichloroethenes (DCEs) and vinyl chloride (VC) often  
accumulate in contaminated aquifers in which PCE or TCE undergo incomplete  
reductive dechlorination. The accumulation of VC is of particular concern  
because VC is a proven human carcinogen. Organisms capable of using  
polychlorinated ethenes as terminal electron acceptors in their energy  
metabolism (e.g., chloridogenic populations) have been isolated over the  
last few years, however, no VC-respiring isolates have been described. An  
anaerobic VC-respiring population, designated as strain BAV1,  
was isolated from a PCE-to-ethene-dechlorinating microcosm obtained from  
the Bachman Road site, a PCE-contaminated aquifer in Oscoda, MI. 16S rRNA  
gene sequencing placed the isolate in the Pinellas group within the  
Dehalococcoides cluster. Strain BAV1 grew in defined  
mineral salts medium with VC as the only available electron  
acceptor. VC (0.83 mM, nominal concentration) was dechlorinated

at rates of up to 57.9  $\mu\text{moles/L/d}$  to ethene, which was not further transformed. Hydrogen was the required electron donor, and the isolate also dechlorinated vinyl bromide, cis-DCE, trans-DCE, 1,1- DCE and 1,2-dichloroethane to ethene. cis-DCE, trans-DCE, and 1,1-DCE (apprx0.8 mM each) were dechlorinated at rates of up to 61.9, 50.5, and 56.0  $\mu\text{moles/L/d}$ , respectively. Dehalococcoides 16S rRNA gene-targeted real-time PCR confirmed chloridogenic growth, and demonstrated that about twice as much biomass was produced when strain BAV1 was grown with cis-DCE compared to growth with VC. This finding indicates that the new isolate captures the energy released from both reductive dechlorination steps. Strain BAV1 failed to grow with PCE and TCE but cometabolized both compounds in the presence of a growth-supporting chloroethene. Strain BAV1 is the first isolate using the priority pollutant VC as a respiratory electron acceptor, and has potential applications in engineered bioremediation approaches.